reporter gene systems, the transcription of which is activated by the mutant regulator in the presence of the effector organic compound.

Applicants' method of generating mutant regulators having expanded or enhanced effector specificity utilizes DNA-based mutagenesis techniques which are specifically targeted only to the sensor domain of the regulator gene, such as mutagenic PCR. In contrast to earlier described methods for generating regulator mutants using chemical mutagenesis techniques, applicants' method eliminates the possibility of generating mutations outside of the sensor domain which could result in increased copy number, altered DNA-binding activity or unproductive regulator-RNA polymerase interaction. The use of chemically-generated mutant regulators having such properties in detection assays may render the results unreliable in view of the fact that any detected transcriptional activation or enhancement cannot definitively be said to have resulted *only* from a change in the effector recognition properties of the regulator protein. The prior art did not teach or suggest the importance of targeted mutation, and arguably taught away from a targeted approach by neglecting to consider the issue.

Applicants' method for generating and screening mutant regulators is also less complex, more efficient and more reliable than the previously described methods. One benefit of targeting mutagenesis to the sensor domain alone is that it is more efficient. There is no lethality to the cells and no effect on the ability of the plasmid to replicate. Thus, one recovers a significantly larger number of mutations in the gene of interest and, as well, one avoids the last few steps of isolating the mutation and re-introducing it into the sensor domain. As described in the application, applicants were able to generate several mutant DmpR regulators having dramatic enhancements in penolic effector recognition relative to wild type DmpR (i.e., up to 60-fold over wild type). The prior art described a single mutant DmpR showing relatively lower increased response to some penolics (i.e., less than 3-fold over wild type).

The invention provides several exemplary mutant DmpR proteins capable of recognizing various substituted phenols at low concentrations in both liquid and soil assays systems. For the convenience of the Examiner, the table below shows the approximate fold-increase in effector recognition observed with several specific DmpR mutants and a number of substituted phenols at defined concentrations. These figures were derived directly from the results shown in Figures 2-7 (as also described on pages 11 and 12 of the specification). Bacteria carrying these mutations showed a generally very high fold-increase in effector recognition across several different phenolic compounds. The observed enhancements often exceeded 20-fold relative to wild type.

INCREASE IN PENOLIC EFFECTOR RECOGNITION – DmpR MUTANT OVER WILD TYPE

MUTANT	PHENOLIC EFFECTOR COMPOUND	APPROXIMATE FOLD INCREASE
DmpR-B21	.0025 mM 2-chlorophenol	60
	.025 mM 2,4-dichlorophenol	20
DmpR-B23	.0025 mM 2-chlorophenol	28
	0.5 ppm2-chlorophenol	24
DmpR-B17#2	.025-.15 mM 4-chloro-3-methylphenol	20-27
	1 ppm2,4-dichlorophenol	6.4
DmpR-B9	4 ppm 2,4-dichlorophenol	6
	2.5 ppm phenol	4.7
DmpR-B31	2 ppm 2-chlorophenol	4
	8 ppm 2,4-dimethylphenol	48
	10 ppm 4-nitrophenol	52
DmpR-D12	2 ppm 2,4-dichlorophenol	10
	10 ppm 4-chloro-3-methylphenol	9
DmpR-D9	0.025 mM 2-chlorophenol	18.9
	0.075 mM 2-nitrophenol	5.6